PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/052035	03.09.2004	05.09.2003

International Patent Classification (IPC) or both national classification and IPC
C12N15/82, C12Q1/48, C12N9/12, A01H5/00

Applicant
CROPDESIGN N.V.

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

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WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/EP2004/052035

Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☒ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-24

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	24
	No:	Claims	1-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-24
Industrial applicability (IA)	Yes:	Claims	1-24
	No:	Claims	

2. Citations and explanations

   see separate sheet

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY (SEPARATE SHEET)

International application No.
PCT/EP2004/052035

Re Item IV.

1. Lack of Unity (Rule 13.1 PCT)
The subject matter of the current claims concerns at least two different problems to be solved. Claims 25-31 relate to the provision of a screening method for the identification of CDK mutants and the CDK mutants of the A-type obtained thereby; whereas claims 1-24 are directed to the provision of methods for modulating the growth characteristics of plants by providing plant specific CDKs of the B-type and recombinantly expressing the same in transgenic plants.
Accordingly, the current application lacks unity a priori.

In the light of the prior art, the following problems and corresponding solutions can be identified:

1st Problem:
The provision of a method to modify plant growth characteristics.
1st Solution:
Method to modify plant growth characteristics by introducing nucleic acid molecules encoding A. thaliana B-type CDKs as characterized by SEQIDs1,3,5 and 2,4,6, respectively.

2nd Problem:
The provision of a screening method for the identification of mutant CDKs.
2nd Solution:
Screening method for the identification of mutant CDKs exhibiting an enhanced CDK activity or of non-active CDKs; nucleotide sequences encoding CDK mutants as represented by SEQIDs 9-13.

In view of the fact that the subject matter of current application concerns at least two different problems to be solved; due to the essential difference between the identified problems and their corresponding solutions, and due to the fact that no

other technical features can be distinguished which, in the light of the prior art could be regarded as special technical features, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the current application within the sense of Rule 13 PCT.

Accordingly, the subject matter was divided into two different inventions:

Invention 1, claims 1-24 completely:
Method of improving the growth characteristics of a plant by increasing the expression of a nucleotide sequence encoding a B-type CDK, by recombinant means, comprising the introduction of a nucleic acid molecule encoding transcription factors, activators, inhibitors, regulatory sequences, ligands or a B-type CDK or variants thereof; said B-type CDKs are characterized by the nucleotide and the respective peptide sequences as in SEQIDs 1,3,5 and 2,4,6; plants obtainable by said methods; construct comprising a nucleic acid sequence encoding a CDK mutant comprising at least one of the amino acid position exchanges shown in Table A or Table B, said nucleic acid sequence characterized by SEQIDs 1,3,5; method for the production of transgenic plants having modified growth characteristics; transgenic plants having modified growth characteristics, the use of a CDK B-type nucleic acid or amino acid in modifying growth characteristics of plants.
A composition comprising a protein represented by SEQIDs 2-6.

Invention 2, claims 25-31 completely:
Screening method for the identification of mutant cyclin dependent kinases (CDKs) exhibiting enhanced CDK activity relative to the corresponding non-mutated CDKs, said method comprising the steps of :
a) providing CDK mutants
b) identifying ICK-non-reacting mutants
c) identifying mutants having cyclin-binding activity
d) yeast complementation assay on resultant mutants
Furthermore, screening method for the identification of non-active CDKs which are

capable of binding ICKs, comprising the steps of:
a) providing CDK mutants
b) identifying ICK binding mutants
c) identifying non-cyclin-binding mutants;

the CDK mutants being provided by the steps of:
a) providing a wild-type CDK
b) mutating said CDK at least at 1 amino acid position;
Mutant CDKS obtainable by said screening methods; Isolated nucleotide sequences encoding CDK mutants and characterized by SEQIDs 9-13; the CDK mutants characterized by SEQIDs 9-13.

The search was limited to the first invention of this PCT application and the opinion will also be limited to the subject matter of the first invention.

Opinion:

1. The following documents are considered relevant for the current application:

D1: WO9841642
D2: Porceddu, A., et al.,2001, JBC, Vol. 276, 39, pp. 36354-36360
D3: Yoshizumi, T., et al., 1999, Plant Cell, 11, 10, pp. 1883-1895
D4: WO0056905
D5: WO0216655

Re Item V.

2. Novelty and **Clarity** (Art. 33(2) and Art. 6 PCT)

2.1 The current application is dealing with the provision of a method for "improving" plant growth through the overexpression of Arabidopsis thaliana-specific cyclin dependent kinases of the B-type (CDKB1;1, CDKB1;2 and CDKB2;2) in transgenic plants.

2.2 As currently drafted, independent claim 1 refers to a very general method of "improving" plant growth characteristics selected from one or more of increased yield, increased growth rate and "modified" architecture". Due to the unclear wording, claim 1 is open to broad interpretation by the reader who has to guess what is meant by "improved plant growth characteristics" and "modified architecture". See also claim 23.
Moreover, claims 7-10 are referring to "B-type CDK" nucleic acids which are characterized by the minimal requirement as to be "capable of hybridizing" to the specifically mentioned SEQIDs. Said wording applies to too many undefined sequences that do not even have to belong to the group of "B-type CDKs" and thus said claim becomes unclear. Prior art documents dealing with the A. thaliana cdc2b-gene fall within the scope of claims 7-9.
Furthermore, it is evident that terms like "B-type CDK" (see claim 1) or "class 1 B-type CDK" or "CDKB1;1" (see claim 5) are not clear to the reader.

2.3 Documents D1-4 all deal with the expression of cyclin dependent kinase-specific nucleic acid sequences belonging to the "B-type CDKs" and due to the broad wording of the claims, said documents are therefore novelty-destroying for claims 1-23 pursuant to Art. 33(2) PCT.

3. **Inventive Step** (Art.33 (3) PCT)

3.1 WO9841642 (D1), which is the closest prior art, discloses the A. thaliana cdc2b-gene ("B-type CDK"), the recombinant overexpression of the same in transgenic tobacco plants and its use in methods to modify plant growth characteristics.
3.2 The difference between D1 and the current application is the use of homologous

"B-type CDK"-specific nucleic acid sequences for the same purpose.

3.3 The problem of the current application is the provision of alternative nucleic acid sequences encoding "B-type CDKs" for modifying plant growth characteristics.
3.4 The solution are nucleic acid sequences encoding the "B-type CDKs" CDKB1;1, CDKB1;2 and CDKB2;2 as characterized by SEQIDs 1,3,5 and 2,4,6, respectively.

3.5 Faced with the identified problem, the person skilled in the art would know how to get hold of already known genes encoding "B-type CDKs" or alternatively how to identify homologous members of the "B-type CDK"-family from A. thaliana.
Generally applicable methods are readily available to the skilled artisan to identify the three "B-type CDKs"-specific sequences encoding CDKB1;1, CDKB1;2 and CDKB2;2 as disclosed in the current application. Moreover, documents D1-D4 teach that "B-type CDKs" have a potential effect on plant growth in general and said documents also teach how to increase the activity of "B-type CDKs" in transgenic plants and how to analyze the effect of this overexpression in the generated plants.

3.6 Consequently, the overexpression of other, alternative "B-type CDK"-specific sequences in transgenic plants is obvious to the skilled artisan and the subject matter of claims 1-24 is not inventive according to Art.33 (3) PCT.